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10/536,495	05/10/2006	Christoffer Bro	BRO2	6559

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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1633	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/536,495	BRO ET AL.	
	Examiner	Art Unit	
	MARIA LEAVITT	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12-13-2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-16* is/are pending in the application.

4a) Of the above claim(s) *14-16* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *25 May 2005* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *05-10-2006*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Status of Claims. Claims 1-16 are pending. Applicant's election with traverse of Group I, claims 1-13, drawn to a metabolically engineered microorganism, in Applicants reply filed on 12-13-2007 is acknowledged. Applicants' election of the following species with traverse is acknowledged: *Saccharomyces cerevisiae* as recited in claims 12 and 14, ethanol as recited in claim 15 and the undesired product glycerol as recited in claim 16.

Response to arguments

On page 1 of Applicants' Response to the Restriction Requirements, Applicants argue that the technical feature of the present claims do define a contribution over Bianchi et al., (2001, Applied Environmental Microbiology, pp. 5621-5625) because Bianchi was given "the Category Code A in the International Search Report indicating that it is only of background relevance. Thus, in the opinion of the ISA/EP, it does not anticipate or render obvious the claims and hence does not deprive them of a unifying special technical feature. While that decision is not binding on USPTO, it is entitled to consideration". Additionally, Applicants argue that the special technical feature which actually links the claims of Groups I and II is that the claimed metabolically engineered micro-organism is a yeast having a "second metabolic pathway characterized by an enzyme activity in excess of a native level in respect of a "third" enzyme catalyzing a non-reversible reaction in which NADP is a co-factor and NADPH is a product". This feature leads to the optimization of the yield of fermentation products by reducing or eliminating the formation of by-products but is neither disclosed nor hinted at in Bianchi et al. Such is not persuasive.

Claim 1 is broadly drawn to a metabolically engineered *S. cerevisiae* " having an operative first metabolic pathway in which a first metabolite is transformed into a second metabolite in a reaction in which NAD is a cofactor for a first enzyme, said reaction step producing NADH, and in which said second metabolite is transformed into at least one further metabolite in a reaction catalysed by a second enzyme and having a second metabolic pathway characterized by an enzyme activity in excess of a native level in respect of a third enzyme catalyzing a non-reversible reaction in which NADP is a co-factor and NADPH is a product. Clearly, the fermentative metabolism of *Kluyveromyces lactis* Strains defective in Pyruvate utilization and transformed with heterologous LDH gene taught by Bianchi et al., implicitly has a first metabolic pathway in which a first reaction metabolite, e.g., Glyceraldehyde 3-phosphate, is transformed into a second metabolite, e.g., 1,3-Bisphosphoglycerate, in a reaction in which a NAD+ is a cofactor for a first enzyme, e.g, Glyceraldehyde 3-phosphate dehydrogenase, and in which the second metabolite is transformed into a on further metabolite, e.g., 3-Phosphoglycerate, absent evidence to the contrary. Moreover, it is unclear how the "second metabolic pathway characterized by an enzyme activity in excess of a native level in respect of a third enzyme catalyzing a non-reversible reaction in which NADP is a co-factor and NADPH is a product' as embraced by claim 1, (e.g., the first substrate level phosphorylation step of the glycolysis) relates to the functional activity of the third enzyme and whether the first metabolite is simultaneously transformed into a second and third metabolite or they are separate metabolic conversions . Further, the art teaches a metabolic engineering glycolytic pathway wherein a functional heterologous **non-phosphorylating NADP-dependent glyceraldehyed-3-phosphate dehydrogenase** was expressed in *E. coli* resulting in net oxidation of Glyceraldehyde

3-phosphate and $NADP^+$ into 3-phosphoglycerate and NADPH by bypassing the first substrate level phosphorylation step of the glycolysis (Valverde et al., FEBS, 1999, 21898, pages 153-158).

Moreover, as indicated in MPEP 1850, PCT Rule 13.2, as it was modified effective July 1, 1992, no longer specifies the combinations of categories of invention which are considered to have unity of invention. The categories of invention in former PCT Rule 13.2 have been replaced with a statement describing the method for determining whether the requirement of unity of invention is satisfied. Unity of invention exists only when there is a technical relationship among the claimed inventions involving one or more special technical features. In the instant case, the special technical feature shared by the Groups is anticipated by the prior art of Bianchi et al., and Valverde et al.

In relation to the rejoining of elected Groups I, drawn to a product and Group II, drawn to the use of said product, it is noted that the MPEP 1893.03(d) states: If an examiner (1) determines that the claims lack unity of invention and (2) requires election of a single invention, when all of the claims drawn to the elected invention are allowable (i.e., meet the requirements of 35 U.S.C. 101, 102, 103 and 112), the nonelected invention(s) should be considered for rejoinder. Any nonelected product claim that requires all the limitations of an allowable product claim, and any nonelected process claim that requires all the limitations of an allowable process claim, should be rejoined. See MPEP § 821.04 and § 821.04(a). Any nonelected processes of making and/or using an allowable product should be considered for rejoinder following the practice set forth in MPEP § 821.04(b).

Claims 14-16 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 12-13-2007.

The restriction is considered proper and made FINAL.

Claims 1-13 are currently under examination to which the following grounds of rejection are applicable.

Notice of Non-Compliant Amendment (37 CFR 1.821 through 1.825)

This application contains sequence disclosures that are encompassed by the definitionsfor nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through1.825 for the reason(s) set forth below.

1. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 C.F.R. 1.821(c). The specification discloses at pages 20-22, Example 6, a nucleotide sequence referred as "Sequencing of GapN". For compliance with the sequence rules, it is necessary to include the sequence in the "Sequence Listing" and identify them with SEQ ID NO. In general, any sequence that is disclosed and/or claimed as a sequence, i.e., as a string of particular bases or amino acids, and that otherwise meets the criteria of 37 CFR 1.821(a), must be set forth in the "Sequence Listing". (see MPEP 2422.03).

Additionally, the following items are required:

2. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e).

3. A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

To be fully responsible for restriction, Applicant is required to comply with the Requirements For Patent Applications Containing Nucleotide Sequence And/Or Amino Acid Sequence Disclosures.

Information Disclosure Statement

The listing of references in the specification is not a proper information disclosure statement. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609.04(a) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the examiner on form PTO-892 has cited the references, they have not been considered.

The information disclosure statement (IDS) submitted on 05/15/2006 has been considered by the examiner.

Drawing Objection

The drawings are objected to under 37 CFR 1.83(a) because they fail to show the following structural details as recited in the specification at page 10, lines 15-21.
"the conversion of glyceraldehyde-3-phosphate into 3-phosphoglycerate is only catalysed by the sequential action of two enzymes, i.e. NAD^+-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (EC 1.2.1.12) and phosphoglycerate kinase (PGK) (EC 2.7.2.3) by conversion of NAD^+ and ADP into NADH and ATP (see figure 1)". Any structural detail that is essential for a proper understanding of the disclosed invention should be shown in the drawing.

MPEP § 608.02(d). Corrected drawing sheets in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Specification objection

The disclosure is objected to because of the following informalities: At page 17, line 23 bridging to page 18, line 1, the specification as filed refers to "the metabolites during anaerobic fermentations is shown in FIG. 3". However, there is not Fig. 3 as part of the drawings filed on 05-25-2005. Moreover, there is not description of Fig.3 at page 11, lines 15-27 (e.g., Brief description of the figures).

Appropriate correction is required.

Claim Objection

Claims 1, 6, and 7 are objected to because of the following informalities: abbreviations such as NADH, GAPN and GAPDH should be spelled out at the first encounter in the claims. Appropriate correction is required.

Claim Rejections - 35 USC § 112- Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claims 1, 6, 7 and 13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite in that it fails to point out what is included or excluded by the claim language.

Additionally claim 1 recites "an operative second metabolic pathway characterised by an enzyme activity in excess of a native level in respect of a third enzyme catalysing a non-reversible reaction". The specification as filed does not teach a definition of "a native level". Hence it is unclear whether "the native level" of the second metabolic pathway refers to the activity of a wild type enzyme with no amino acids substitutions or to the functionality of a second enzyme in the presence of the cofactor in relation to the activity of a third enzyme. Moreover, it is unclear whether the first metabolite is simultaneously transformed into a second and third metabolite or they are separate metabolic conversions. Thus the metes and bounds are of the claim are unclear.

Claims 6, 7 and 13 use parentheses to comments on or qualify part of the sentences. It is unclear whether the limitations in parentheses are meant to be limitations in the claims or

whether they are only suggestions/examples. As such, the metes and bounds of the claims cannot be determined.

For the purpose of a compact prosecution, claim 1 is interpreted as having a third enzyme catalyzing a non-reversible reaction in which NADP is a cofactor and NADPH is a product said third enzyme able to transform a fist metabolite into a second metabolite without the involvement of a second enzyme wherein said metabolic conversion leads to reduce formation of NADH and ATP.

Claim Rejections - 35 USC § 103

Claims 1-13 are rejected under 435 U.S.C. 103(a) as being unpatentable over of Nissen et al., (Metabolic Engineering, 2000, 2: pages 69-77) in view of Valverde et al., (FEBS, 1999, 21898, pages 153-158).

The present invention is drawn to metabolically engineered *S. cerevisiae* expressing a cDNA comprising the *Streptococcus mutants* GapN gene on a multicopy plasmid which encodes for the **non-phosphorylating $NADP^+$- dependent glyceraldehyed-3-phosphate dehydrogenase (GAPN)** (p.11 lines 29-32). GAPN catalyses the irreversible oxidation of glyceraldehyde-3-phosphate and $NADP^+$ into 3-phosphoglycerate and NADPH. The reaction catalysed by GAPN yields one NADPH instead of one NADH and one ATP when comparing with the total reaction catalysed by **NAD^+-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH)** and phosphoglycerate kinase (PGK) (p. 10, lines 14-32). Moreover, the specification discloses that anaerobic growth of *S. cerevisiae* on a fermentable sugar generates surplus amounts of NADH, and this results in the formation of by-products, primarily glycerol (p. 4, lines 14-19). Glycerol formed by *S. cerevisiae* during anaerobic growth to

maintain the cytosolic redox balance is a redox problem, so by introducing gapN into *S. cerevisiae* the following metabolic modification are observed: the production of glycerol is reduced by one molecule for each molecule of glyceraldehyde-3-phosphate that is converted via GAPN and the flux redirected to ethanol and/or biomass thereby increasing the ethanol yield (p. 12, lines 19-32).

Nissen et al., teaches a metabolically engineered *S. cerevisiae* wherein reduced formation of surplus NADH and an increased consumption of ATP in biosynthesis results in decreased glycerol yield. Specifically, Nissen et al., describes a mutant *S. cerevisiae* in which *GLN1*, encoding glutamine synthetase, and *GLT1*, encoding glutamate synthase, were overexpressed, and *GDH1*, encoding the NADPH-dependent glutamate dehydrogenase, was deleted (p. 70, col. 2, paragraph 4). The *S. cerevisiae* mutant exhibited an altered cofactor consumption in the ammonia assimilation resulting in consumption of 1 mol of NADH and ATP per mole of glutamate instead of 1 mol of NADPH leading to a reduction surplus formation of NADH resulting a 49% reduction of glycerol formation and a 6% increase in ethanol production (p. 70, col. 2, last paragraph bridging to p. 71, col. 1, first paragraph; p. 75, Table 2). It is noted that the metabolically engineered *S. cerevisiae* implicitly has a first metabolic pathway in which a first reaction metabolite, e.g., Glyceraldehyde 3-phosphate, is transformed into a second metabolite, e.g., 1,3-Bisphosphoglycerate (1,3-BPGA), in a reaction in which a NAD+ is a cofactor for a first enzyme, e.g, Glyceraldehyde 3-phosphate dehydrogenase, and in which the second metabolite is transformed into a on further metabolite, e.g., 3-Phosphoglycerate. Additionally, Nissen et al., states that a number of byproducts are are formed during an anaerobic fermentation of *S. cerevisiae* of which Glycerol is the most important, consuming up to 4% of the carbon

source in industrial fermentation. Moreover out Nissen et al., discusses that by eliminating formation of Glycerol it is possible to increase the yield of ethanol (p. 69, col. 2, paragraph 1) (Current claims 1, 2, 3, 4, 7, 10-12).

Nissen et al., does not specifically teach a metabolic engineering *S. cerevisiae* with reduced formation of NADH and ATP resulting from the enzymatic activity a non-phosphorylating dehydrogenase.

However, at the time the invention was made, Valverde et al., discloses a metabolic engineering glycolytic pathway wherein a functional heterologous **non-phosphorylating NADP-dependent glyceraldehyed-3-phosphate dehydrogenase** (**GAPN**) was expressed in *E. coli* resulting in net oxidation of Glyceraldehyde 3-phosphate and $NADP^+$ into 3-phosphoglycerate and NADPH. Moreover, Valverde et al., discloses that **GAPN** bypasses the first substrate level phosphorylation step of the glycolysis i.e., conversion of Glyceraldehyde 3-phosphate into 1,3-Bisphosphoglycerate (p. 152, col. 2, paragraph 2) (Current claims 5 and 6). Moreover, Valverde et al., teaches the construction of the plasmid pFVNP1, which contains the gene encoding the **GAPN** under the control of a strong *trc* promoter. Said plasmid was used to transform *E. coli* resulting in functional **GAPN** (p. 155, col. 1, paragraphs 1 and 2) (Current claims 8, 9 and 13). Thus, in contrast to the cytosolic NAD-dependent GAPDH which is responsible for the first substrate level phosphorylation step of the glycolysis, rendering 1,3-Bisphosphoglycerate, the substrate of 3-Phosphoglycerate kinase which eventually produces 3-Phosphogycerate and ATP, **GAPN** exhibits a metabolic engineering catabolic glycolytic pathway with no net substrate level phosphorylation (p. 153, col. 2, paragraph 3; page 157, Fig. 4). Clearly, the net catabolic yield of the glycolytic pathway catalyzed by **GAPN** is one NADPH

as compared to one NADH and one ATP produced by the reaction catalyzed in the successive metabolic transformation by glyceraldehyde 3-phosphate dehydrogenase and phosphoglycerate kinase .

Therefore, in view of the benefits of metabolic engineered *S. cerevisiae* wherein reduced formation of NADH and ATP results in decreased glycerol yield and enhanced ethanol production as taught by Nissen, it would have been *prima facie* obvious for one of ordinary skill in the art to reduce the surplus of NADH by metabolically reducing the amount of NADH produced in fermentative pathways involved in ethanol production as taught by Valverde et al., particularly because Nissen teaches optimization of ethanol production and reduction of glycerol formation by a reduction of surplus formation of NADH. The manipulation of previously identified enzymes in metabolic pathways in fermenting yeast strains for the production of ethanol is within the ordinary level of skill in the art. One of ordinary skill in the art would have been motivated to clone and express a functional **GAPN** in *S. cerevisiae* to render 3-phosphoglycerate from glyceraldehyde 3-phosphate with a reduced net formation of NADH by bypassing the first substrate level phosphorylation of glyceraldehyde 3-phosphate in fermenting yeast with a reasonable expectation of success.

Conclusion

Claims 1-13 are not allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria Leavitt whose telephone number is 571-272-1085. The examiner can normally be reached on M-F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, Ph.D can be reached on (571) 272-0739. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

To aid in correlating any papers for this application, all further correspondence regarding his application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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/Maria Leavitt/

Maria Leavitt, PhD
Examiner, Art Unit 1633

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	10/536,495	BRO ET AL.	
	Examiner	Art Unit	
	MARIA LEAVITT	1633	